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COMPLEMENT FIXATION IN ACUTE RHINITIS *

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Tunnicliff¹ discovered in the early stages of acute rhinitis an anaerobic bacillary or spirochetal organism in the nose which has been designated tentatively as *Bacillus rhinitis*. The organism produced acute coryza experimentally in the human subject, and in the early stages of the experimental, as well as of the natural, disease Tunnicliff found the opsonin for the organism to be below normal, the index rising as the infection subsided. In view of these results it seemed of interest to determine whether the sera of cases of acute rhinitis contain any complement-fixing antibodies for this organism.

METHOD

The following bacteria were used as antigens: *Bacillus rhinitis*, suspensions of either single strains or of a mixture of six strains; *B. fusiformis*; *staphylococcus*; *streptococcus*, hemolytic and viridans; *pneumococcus*; *Micrococcus catarrhalis*; *B. influenzae*; *B. mucosus*; diphtheroid bacilli. The strains of *B. rhinitis* were furnished by Dr Tunnicliff.

The strains of the *bacillus rhinitis* and of the fusiform bacillus were grown anaerobically for forty-eight to seventy-two hours on goat blood agar or in ascites broth. The other bacteria were grown aerobically for twenty-four hours on agar slants. In each case, suspensions were made in salt solution and heated at 56 C. for one hour.

The anticomplementary unit of each antigen was determined carefully and in the tests $\frac{1}{2}$ to $\frac{1}{16}$ of this unit was used.

The sera from the patients were heated to 56 C. for thirty minutes and 0.02 c.c. of the undiluted serum constituted the unit.

The antisheep hemolytic system was used, 0.1 c.c. of a dilution 1:10 of fresh guinea-pig serum being the complement unit, and the quantity of sheep blood used was 0.1 c.c. of a 5 percent suspension. The amboceptor was titrated separately for each series of tests, and twice the hemolytic unit was used in each test. Whenever natural

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1. Jour. Infect. Dis., 1913, 13, p. 283.

antisheep amboceptor was encountered in the serum to be tested, amboceptor enough to make two hemolytic units only was added.

The serum, the antigen, the amboceptor, the complement, and the sheep blood and the hemolytic system were controlled in each series of tests.

In making the tests the antigen, the serum to be tested, and the complement were incubated at 37 C. for one hour. The amboceptor and corpuscles were then added and the mixture incubated for thirty minutes or longer according to the controls.

THE SERA OF PERSONS INOCULATED WITH THE BACILLUS RHINITIS

The sera of three persons each of whom had received several doses of autogenous vaccines of the bacillus rhinitis were available. In each case the serum caused fixation of complement with the autogenous antigen and a less marked positive result with the other two antigens of the bacillus rhinitis and with the polyvalent rhinitis antigen. It was possible to make frequent tests of the serum of one of these persons in whom the inoculations were discontinued in the middle of November, 1914. At this time, the serum gave a markedly positive result with autogenous antigen. The fixation became less and less marked and in February, 1915, there was no fixation. Three inoculations of vaccines were now made, and the serum again gave a strongly positive reaction.

TABLE 1

| COMPLEMENT FIXATION BY THE SERA OF PATIENTS INOCULATED WITH BACILLUS RHINITIS | | Results |
|---|------------------------------|---------|
| Serum | Antigen | |
| Case 1..... | B. rhinitis 1..... | +++ |
| | B. rhinitis 2..... | ++ |
| | B. rhinitis 3..... | + |
| | B. rhinitis, polyvalent..... | + |
| | B. fusiformis | 0 |
| Case 2..... | B. rhinitis 1..... | +++ |
| | B. rhinitis 2..... | +++ |
| | B. rhinitis 3..... | +++ |
| | B. rhinitis, polyvalent..... | +++ |
| | B. fusiformis | 0 |
| Case 3..... | B. rhinitis 1..... | ++ |
| | B. rhinitis 2..... | ++ |
| | B. rhinitis 3..... | +++ |
| | B. fusiformis | 0 |

B. rhinitis 1 was originally isolated from Case 1; B. rhinitis 2 from Case 2; B. rhinitis 3 from Case 3.

The sign +++ = complete inhibition; ++ = partial inhibition; + = slight inhibition; 0 = no inhibition.

In these tests *B. fusiformis* antigen was included in each case because of the resemblance between this organism and *B. rhinitis*. The results, however, indicate no relationship.

SERA OF PATIENTS WITH ACUTE RHINITIS (ACUTE COLDS)

The sera of nineteen cases of acute rhinitis have been examined. In most cases several strains of the bacillus rhinitis were used as antigens. There were slight variations in results with different strains, but in no case did the serum give a positive reaction with one or more strains and a negative reaction with any of the other strains. Of the nineteen cases studied, seven gave strongly positive results during some stage of the attack; four less strongly positive; and six only slightly positive. The remaining two cases gave negative results. It was difficult to obtain exact information regarding the duration of the attack when serum was obtained. However, the strongly positive sera were obtained as a rule between the third and eighth days of the attack. During the earliest stages the serum usually caused no fixation, or only slight fixation, of complement. The results in the cases in which serum was obtained several times indicate that the period during which the serum will cause fixation with the bacillus rhinitis as antigen is rather short. In one case the serum remained positive for six weeks and in another case for two weeks, but usually the power of fixation was lost within a few days. In five instances the serum had been examined before acute rhinitis developed, with negative results. During the attack the serum in two cases became strongly positive, one a little less strongly positive, and two feebly positive.

The sera of three patients with acute rhinitis giving positive fixation with the bacillus rhinitis were tested also with the other antigens mentioned in describing the method used in this work, and one serum gave a slight fixation with the pneumococcus antigen, altho there were no pneumococci present in cultures made from the nose. The patient had had pneumonia two years previously. Two of the sera gave a feeble or slight fixation with pseudodiphtheria antigen; one of these two sera gave an equally positive result before the cold. In eight cases in which complement fixation was obtained with rhinitis antigen, tests were made also with antigens of whatever other bacteria were found in smears or cultures from the nose, but with negative results.

THE SERA OF NORMAL PERSONS AND OF PATIENTS WITH DISEASES
OTHER THAN RHINITIS

Tests were made for complement fixation with the bacillus rhinitis as antigen with the sera of thirteen persons apparently in normal condition and without any history of a recent attack of rhinitis. In each

case the result was frankly negative. The sera of patients with scarlet fever, typhoid fever, pneumonia, rheumatism, diphtheria, Vincent's angina, measles, and syphilis were tested in a similar way with negative results in every case except one case of measles which gave a feebly positive result.

TABLE 2
COMPLEMENT FIXATION BY THE SERA OF PATIENTS WITH ACUTE RHINITIS

| Number | Approximate Duration in Days of Attack | Results |
|--------|--|---------|
| 1 | 3 | +++ |
| 2 | 7 | ++ |
| 3 | 3 | +++ |
| 4 | 3 | +++ |
| 5 | 2 | + |
| 6 | Several | +++ |
| 7 | Several weeks | + |
| 8 | 2 | 0 |
| 9 | 3 | + |
| 10 | Several | ++ |
| 11 | Several | ++ |
| 12 | Repeated attacks | ++ |
| 13 | 2 | + |
| 14 | 2 | + |
| 14 | 4 | +++ |
| 15 | 2 | + |
| 16 | 2 | 0 |
| 16 | 4 | + |
| 16 | 5 | 0 |
| 17 | 2 | +++ |
| 18 | 4 | ++ |
| 18 | 8 | +++ |
| 19 | 2 | 0 |
| 19 | 4 | 0 |

The sign +++ = complete inhibition; ++ = partial inhibition; + = slight inhibition; 0 = no inhibition.

TABLE 3
COMPLEMENT FIXATION BY THE SERA OF PATIENTS WITH ACUTE RHINITIS IN THE PRESENCE OF VARIOUS ANTIGENS

| Case | Antigens | | | | | | | | | Pseudo-Diphtheria Bacillus |
|------|-------------|-------------|---------------|-------------------------|------------------------|------------|---------------|---------------|----------------|----------------------------|
| | B. Rhinitis | Pneumococci | Staphylococci | Streptococcus Hemolytic | Streptococcus Viridans | B. Mucosus | B. Fusiformis | B. Influenzae | M. Catarrhalis | |
| 1 | +++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + |
| 2 | ++ | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | ++ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | + |
| 4 | +++ | | | | | | +++ | | | |
| 5 | +++ | | | | | | 0 | | | |
| 6 | + | | | | 0 | | | | | 0 |
| 7 | +++ | 0 | 0 | | 0 | | | 0 | | 0 |
| 8 | + | 0 | | | 0 | | | | | 0 |
| 9 | 0 | 0 | 0 | | | | | | | 0 |
| 10 | + | 0 | 0 | 0 | 0 | 0 | | | | 0 |

The sign +++ = complete inhibition; ++ = partial inhibition; + = slight inhibition; 0 = no inhibition.

CONCLUSIONS

With the use of the bacillus rhinitis as antigen, fixation of complement is obtained with the sera of persons with acute rhinitis and of persons injected with the bacillus after it is killed by heat.

The fixation is most marked a few days after the onset of the infection and lasts only a short time.

Sera of normal persons and of patients with various infectious diseases do not give complement fixation with the bacillus rhinitis.

The sera of patients with acute rhinitis do not give fixation of complement, except occasionally when suspensions of various bacteria (pneumococci, staphylococci, streptococci, influenza bacillus, fusiform bacillus, pseudodiphtheric bacilli, etc.) ordinarily regarded as closely associated with rhinitis, if not the actual cause thereof, are used as antigens.

These results indicate that the bacillus rhinitis bears a specific relationship to acute rhinitis as ordinarily observed in this region.